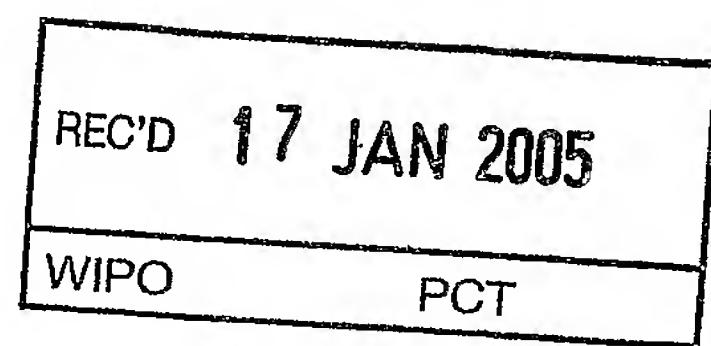# PRV

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(71) *Sökande*   *Bioactive Polymers AB c/o Lunds Universitet, Lund*
     Applicant (s)   *SE*

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SÖKANDE: BIOACTIVE POLYMERS AB   Huvudfaxen Kassan

UPPFINNINGENS BENÄMNING: SURFACE PROTECTION OF EXPOSED
                          BIOLOGICAL TISSUES

5

       The present invention refers to surface protection of
exposed surfaces of biological tissues. More specifically,
10 the invention refers to a cationic polymer and an anionic
polymer in combination for use in human and/or veterinary
medicine for surface protection of exposed surfaces of
biological tissues, especially for preventing fibrinous and
fibrous adhesions of tissues.

15   Tissue surfaces need protection depending on the type
and extent of wearing that they normally are subjected to.
Most tissues within the body are thus covered by a cell
membrane bilayer that mainly consists of lipids and
proteins. However, epithelial surface tissues of respira-
20 tory, gastrointestinal, and to some extent the genitouri-
nary tracts, are normally exposed to a rather harsh
environment. These epithelial surfaces are further covered
by a mucus layer having viscoelastic and pronounced pro-
tecting properties. Such tissues as synovia and
25 mesothelium, on the other hand, are not protected by mucus
since they are not exposed to drastic conditions of the
same magnitude.

       In addition, the vital epithelial tissues, such as
blood vessels or blood organs, are coated with mucous,
30 serous, synovial and endothelial membranes so that they can
function independently of each other. The peritoneal,
pericardial and pleural membranes consist of a single layer
of mesothelial cells, which is covered with a thin film of
peritoneal fluid. The components of the membranes as well
35 as the covering layer of fluid have several functions, e.g.
lubrication of the enclosed organs, unrestricted mobility
being provided.

The protective epithelial membrane is very thin and comprises a delicate layer of connective tissue covered with a monolayer of mesothelial cells and only one or a few bilayers of mainly phospholipids. This makes such tissues
5 as synovia and mesothelium especially vulnerable to infection and trauma. When such a membrane is exposed to a physical, chemical or microbial challenge, many potent substances are often released in response thereto, which are harmful to the membrane. The structure and function of
10 the membrane is consequently easily destroyed in connection with trauma, ischemia, and infection. After an irritation of the stress-sensitive membrane, e.g. only by the desiccation or abrasion of the membrane surfaces during surgery, it will rapidly be covered with a fibrin clot.
15 Since the plasminogen activating activity (i.e. the fibrinolytic capacity) is reduced after trauma, the fibrin clots will later on become organized as fibrous adhesions, i.e. small bands or structures, by which adjacent serous or synovial membranes adhere in an abnormal way. Surgical
20 operations, infection or inflammation in those parts of the body, which are coated with serous or synovial membranes, can result in adhesive inflammation regardless of the size of the affected area. The adhesions between vital epithelial tissues are formed within the first few days
25 following surgery trauma or infection and may be observed not only in particular portions of the body but in all vital tissues. Such adhesions between for example contact zones intestines or intestines and the abdominal wall are the result of the often unnoticed tissue damage as desic-
30 cation and they occur for various reasons, including mechanical and chemical stimulations of vital tissues accompanying surgical manipulations, postoperative bacterial infection, inflammation or further complications.

Adhesion of vital epithelial tissues, large or small,
35 may be observed in most surgical fields. It has been repor-

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ted that of all patients undergoing abdominal surgery at
one hospital over a four-year period, 93 % were found to
have adhesions from previous operations. In addition, in a
10 year period there will be a need of adhesion prevention
5 in about 20% of all surgical operations, which corresponds
to more than 1 million operations annually on each major
continent.

However, the postsurgical adhesions obtained are the
result of a natural wound healing response of tissue damage
10 occurring during surgery. Numerous factors play a role in
peritoneal wound healing and the development of adhesions.
Among others are peritoneal macrophages known to have an
important role in initial peritoneal repair.

Thus, while waiting after surgery for the body to
15 produce new protective layers it is important to supply the
corresponding protection from the outside to exposed epi-
thelial surfaces in an effective way. Furthermore, it is
important to prevent or reduce the infection and/or the
inflammation obtained after surgery as well as the accompa-
20 nying fibrin formation.

Various bioactive materials and macromolecules have
been reported to decrease the extent of postoperative
abdominal adhesions. Likewise, a number of methods for
limiting the formation of surgical adhesion have been
25 studied with some encouraging but often ambiguous results.
However, most efforts made to avoid or reduce postoperative
peritoneal adhesions have finally been abandoned. Among the
methods used prevention of fibrin formation, reduction of
fibrin formation, surface separation, and surgical
30 techniques can be mentioned.

Numerous investigations have been carried out in
which barriers are placed at a site of injury in order to
prevent fibrin bridge formation between the injured tissue
and neighboring organs. Such barriers include resorbable
35 materials, such as enzymatically degradable oxidized re-

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generated cellulose, and slowly dissolving physiochemically crosslinked hydrogels of the Pluronic™ type.

Most methods of surface protection of exposed
epithelial surfaces, whereby a postsurgical adhesion

5   formation is limited, have also focused on providing wound separation by placing a material between the tissues. In addition, several types of viscous polymer solutions such as polyvinylpyrrolidone, sodium carboxymethyl cellulose, dextrans, and hyaluronic acid have been added before and/or

10 at the end of surgery in order to control the wound healing events after the occurrence of the presumed tissue in-juries. These solutions are supposed to act by increasing the lubrication and preventing the fibrin clots from adhering to other surfaces or by mechanically separating

15 damaged tissues while they heal.

The employed polymeric solutions are mainly based on the viscosity of the high molecular weight polymer, which is intended to increases with increasing concentration. The polymer is often a polysaccharide as in US 4,994,277, in

20 which a viscoelastic gel of biodegradable xanthan gum in a water solution for preventing adhesions between vital tissues is described. However, the major disadvantage of these polymers, when used for reducing for example peri-toneal adhesions as protective coatings during surgery or

25 surface separation agents after surgery, is that they do not significantly reduce adhesions because of their short residence time in the peritoneal cavity. The result is that subsequent surgeries have to be performed on the patient.

Less viscous polymer solutions have been used as a

30 tissue protective coatings during surgery in order to maintain the natural lubricity of tissues and organs and to protect the enclosing membrane. Precoating for tissue pro-tection and adhesion prevention includes coating tissues at the beginning of surgery before a significant tissue manip-

35 ulation and irritation can occur and continuously through-

5

out the operation so that a protective coating can be main-
tained on the tissues.

US 5,366,964 shows a surgical viscoelastic solution
for promoting wound healing, which is used in direct
5 contact with cells undergoing wound healing. The solution
is intended for cell protection and cell coating during
surgery and comprises one or several polymeric components.
Hydroxypropylmethyl cellulose and chondroitin sulphate are
supposed to lubricate the tissue, while sodium hyaluronate
10 would provide viscoelastic properties to the solution.

Several agents of today for treating postsurgical
adhesions contain hyaluronic acid. For example US 5,409,904
describes solutions which reduce cell loss and tissue
damage intended for protecting endothelial cells during
15 ophthalmic surgery. The compositions used are composed of a
viscoelastic material comprising hyaluronic acid,
chondroitin sulphate, modified collagen, and/or modified
cellulose. In WO 9010031 a composition is described for
preventing tissue adhesion after surgery containing dextran
20 and hyaluronic acid act which substances are supposed to
act synergistically. In WO 9707833 a barrier material for
preventing surgical adhesions is shown, which comprises
benzyl esters or covalently crosslinked derivatives of
hyaluronic acid.

25   A hyaluronic acid based agent manufactured by
Pharmacia under the trademark Healon and originally in-
tended as an intraocular instillation has been found to the
most effective agent up to now. However, hyaluronic acid is
isolated from cock's crests and is thus very expensive as
30 well as potentially allergenic even in small quantities and
even more for large surfaces such as the peritoneum which
has an area of about two m^2.

In WO 9903481 a composition for lubricating
and separating tissues and biological membranes from
35 adjacent membranes or adjacent cells or tissues is shown,

which comprises a hydrophobised polymer formed from a biologically acceptable water-soluble cationic polymer carrying covalently bound hydrophobic groups.

5 Likewise, water-insoluble biocompatible compositions are shown in EP 0,705,878, which comprise a polyanionic polysaccharide combined with a hydrophobic bio-absorbable polymer.

In US 6,235,313 a variety of polymers were compared for adhesive force to mucosa surfaces. Negatively charged
10 hydrogels, such as alginate and carboxymethyl cellulose, with exposed carboxylic groups on the surface, were tested, as well as some positively-charged hydrogels, such as chitosan. The choice was based on the fact that most cell membranes are actually negatively charged. However, there
15 is still no definite conclusion as to what the most important property is in order to obtain good bioadhesion to the wall of the gastrointestinal tract. For example, chitosan is considered to bind to a membrane by means of ionic interactions between positively charged amino groups
20 on the polymer and negatively charged sialic acid groups on the membrane. Thus, polycationic molecules, such as chitosan and polylysine, have a strong tendency to bind to exposed epithelial surfaces since these generally have a negative net charge.

25 A main drawback of both these cationic molecules is that they exhibit toxic effects. For example, polylysine is considered to act as an inhibitor of the calcium channel by producing a conformational change, thereby inhibiting transmembrane ion fluxes.

30 The object of the invention is to provide a new approach of protecting exposed epithelial surfaces of a mammal, whereby the above-mentioned problems according to the state of the art are avoided or eliminated.

According to the invention, an effective amount of a
35 water soluble cationic polymer, and a pharmaceutically

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acceptable carrier, is used in the manufacturing of a first
drug in combination with an effective amount of a water
soluble anionic polymer, and a pharmaceutically acceptable
carrier, in the manufacturing of a second drug, which drugs
5 are to be administered in sequence to a mammal for surface
protection of exposed tissue surfaces.

The invention also refers to a kit comprising:

(a) a first drug comprising an effective amount of
a water soluble cationic polymer in a pharmaceutically
10 acceptable carrier;

(b) a second drug comprising an effective amount of a
water soluble anionic polymer in a pharmaceutically
acceptable carrier; and

(c) means for administering the first and the second
15 drug in sequence to a mammal for surface protection of
exposed epithelial surfaces.

Without wishing to be bound to any theory, it is
believed that the cationic polymer first binds to a
membrane, and the anionic polymer subsequently binds to
20 the cationic polymer, a complex between the cationic
polymer and the anionic polymer being formed *in situ*.

It was surprisingly found that the toxic effects of
the polycationic molecules could be eliminated by their
fixation or immobilization in a "complex" with polyanionic
25 macromolecules when these molecules were added immediately
after the cationic molecules had bound to the exposed
surface. Thus, the surface layer of bound cationic mole-
cules are immobilized or "inactivated" by the subsequent
administration of an anionic polymer. The cationic and
30 anionic polymers can also be added to the exposed surface
as a single entity, however with a reduced effect.

Furthermore, the anionic polymer and the cationic
polymer in solution interact to significantly increase the
viscosity of the polymeric material between for example
35 membranes. This results in that the desired effect will

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remain and last for a longer time. An attachement of the material and an almost hard interface is obtained.

In fact, the material formed from the cationic and anionic polymers has a low solubility or is almost in-
5 soluble in dependence of the polymers used. The material formed effectively decreases experimental, postsurgical peritoneal adhesions by penetrating into the wound and isolating the same from the abdominal cavity. The material mechanically closes the wounds, rapidly covers other
10 peritoneal surfaces and accumulates selectively around the injured site. Mesothelial cells can then grow over the wound during healing.

In addition, it was found that the material formed on the exposed epithelial surfaces had excellent biodegrad-
15 ability properties. After one month of administration the deposited material disappears and only in few cases can a minimal residual material be detected on intraabdominal postsurgical wounds.

In the drugs to be used according to the invention
20 the water soluble ionic polymers are to be administered in pharmaceutically acceptable carrier. Such carriers are well known to those skilled in the art. Preferably, distilled water or buffered aqueous media are used, which contain pharmaceutically acceptable salts and buffers. Suitable
25 salt solutions are PBS, PBSS, GBSS, EBSS, HBSS, and SBF.

The water soluble cationic polymer to be used according to the invention can be a natural polymer, such as a polysaccharide, a protein, or a polypeptide.

The polysaccharide can be chitosan or a cationic
30 derivative of cellulose or starch.

A suitable protein, which can be used as a natural cationic polymer, is lysozyme.

However, it is preferred the water soluble cationic polymer is a polypeptide, especially a polyamino acid.

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Examples of cationic polyamino acids are polylysine, polyarginine, and polyhistidine.

The water soluble cationic polymer can also be a synthetic polymer.

5   The drug for administration of the water soluble cationic polymer should comprise 0.5-5 wt% of the same in the pharmaceutically acceptable carrier.

Likewise, the water soluble anionic polymer is a natural polymer that can be a polysaccharide, a protein, a

10   polypeptide, or a polynucleotide. Suitable anionic polysaccharides are xanthan as well as alginic acid, hyaluronic acid, and polygalacturonic acid, or their salts. Anionic derivatives of cellulose and starch can also be used, such as carboxymethyl cellulose.

15   Insoluble cellulose and glucans can be derivatized by means of for example phosphorylation, sulfation, or amination to impart solubility to the natural polymer. Examples of such water soluble anionic polymers are dextran sulfate, cellulose sulfate, and sulfopropyl cellulose.

20   Alternatively, the anionic polymer can be N,O-carboxymethyl chitosan (NOCC), which has structural similarities with hyaluronic acid.

However, it is preferred the water soluble anionic polymer is a polypeptide, especially a polyamino acid.

25   Examples of anionic polyamino acids are polyglutamate and polyaspartate.

The drug for administration of the water soluble anionic polymer should comprise 0.5-5 wt% of the same in the pharmaceutically acceptable carrier.

30   It is preferred that the anionic polymer is administered after the cationic polymer has been administered. This, of course, also applies for the corresponding drugs. Preferably, the time span between the administrations should not exceed 10 min, most preferably less than 5 min.

10

The amount of anionic polymer should be administered
in a stoichiometric excess relative the amount of cationic
polymer. Preferably, the amount of anionic polyamino acid
is 1.5-2 times the amount of cationic polyamino acid with
5   reference to the number of anionic amino acid residues in
relation to the number of cationic amino acid residues.

EXAMPLES
The invention will now be further described and illu-
10   strated by reference to the following examples. It should
be noted, however, that these examples should not be con-
strued as limiting the invention in any way.

Example 1. Adhesion prevention.
15   A reproducible and standardized rat and rabbit model
was adopted. Forty eight female MRI mice weighing about 25-
30 g were used to induce the adhesions and forty two for
further tests. The animals were kept under standardized
conditions and had free access to pellet and tap water.
20   Anesthesia was induced by ketamine 150 mg/kg
(Ketalar, Parke Davis) and zylazine 7.5 mg/kg (Rompun,
Bayer Sverige AB) intramuscular injection. After dis-
infection, a 25 mm long midline laparotomy was performed.
Both peritoneal surfaces of the lateral abdominal wall were
25   exposed, and 2x15 mm long sharp incisions were performed at
the same distance from the midline, including the muscles.
The wounds were immediately closed with 2x4 single sutures
at equal distances by using 5.0 polypropylene (Prolene,
Ethicon, Johnson & Johnson). The midline laparotomy was
30   closed in two layers with a continuous 5.0 polypropylene
suture. At the evaluation time an overdose of anesthetic
was administered, the abdomen was totally opened through a
U-shaped incision with its base to the right. The lengths
of the adhesions were measured on both sides using a metal
35   caliper, and data was expressed as percent wounds covered
by adhesions.

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Aqueous solutions of 0.5% poly-L-glutamate, and poly-L-lysine were freshly made on the day of the experiment and stored in refrigator until used. FITZ-labeled polylysine was mixed with polylysine in a proportion of 1:10 (wt). All

5   chemicals and cell culture substrates were purchased from Sigma-Aldrich, St Louis, USA; fluorescent microparticles (Nile Blue Labeled) were bought from Microparticles GmbH., (Berlin, Germany).

The animals were divided randomly into 4 groups based

10 on the treatment and the evaluation time. The control groups were intraperitoneally injected with 2 ml physiologic sodium chlorine solution. Two treatment groups received 1 ml poly-L-lysine solution and 5 min later 1 ml poly-L-glutamate solution. One of the control and treatment

15 groups (2x14 animals) was sacrificed one week after surgery and the lengths of the adhesions were calculated. The remaining two groups (2x10 animals) were kept for four weeks before they underwent the evaluation process.

The Kruskal Wallis test was used to determine the

20 difference in adhesion amount among the different treated groups and the Mann Whitney U test was used to compare the individual groups.

A significant decrease in adhesion development was detected both one week and one month after the peritoneal

25 challenge (**$p \leq 0.001$) compared to the corresponding controls (Mann-Whitney U test). A marked (22%) though not significant ($p = 0.235$) decrease was obtained after one month between the control groups, while there were no difference between the treated groups by that time.

30 No adhesions were found which were related to a heavy compound deposit in different locations from the wound itself. After 24 h, the animals that had been given both poly-L-lysine and poly-L-glutamate exhibited a massive protecting layer over the periotoneal wound, and thin film

35 at the rest of peritoneal surface. However the FITZ-labeled

compound was only visible in the wound one day later and was detectable both over and inside the wound. The deposit was gradually rebuilt until the end of the 6 day observation period.

Example 2. Phagocytosis and particle ingestion index.

The time course of the phagocyte function was tested in vitro on peritoneal resident macrophages from mice after 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h, 12 h, 16 h, and 24 h incubation with poly-L-lysine + poly-L-glutamate ($40 \mu g/ml$) and/or fluorescent particles (1 μm).

Macrophage samples were taken by abdominal lavage with 10 ml ice cold DMEM-solution. The samples in medium were immediately centrifuged at 1200 rpm for 10 min. The cells were resuspended in DMEM containing 10% FBS and penicillin/streptomycin and then plated on 48 wells cell culture plates; $5x10^5$ cells in each well. After 1.5 h non-adherent cells were washed away, particles (100/cell) together with test drugs (poly-L-lysine + poly-L-glutamate) were added in a dose of $40 \mu g/ml$ to 12x5 wells, and particles only were added to the remaining 12x5 wells. Moreover negative controls were performed at each time point. The cells were incubated (37°C, 5% CO_2) and detached and fixed at the evaluation time by using 250 μl 5 mM EDTA and an equal volume of 2% paraformaldehyde. FACS analysis (FACScan, Becton Dickinson, San Jose, CA) was made, when cell size (forward scatter, FSC), granularity (side scatter, SSC) and fluorescence intensity (in FL3 channels) were recorded of $1.5x10^4$ cells in each measurement. In manually defined gates the ratio phagocyting cells/total macrophages was expressed in percent as mean of data from five wells at each time and treatment group (control and poly-L-lysine + poly-L-glutamate).

The non-treated cells incorporated more particles. Thus, the maximum plateau (median) level of their fluorescence intensity (FL3) and SSC was set as 100%. All

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measurements were expressed as the median percentage of the plateau level and termed particle ingestion index, since it refers to the amount of particles ingested.

     The Mann Whitney U test was used to check the plateau
5 of phagocytosis and the Wilcoxon Signed Ranks test was used to test the difference in the phagocytosis and particle ingestion index between the treatment pairs (control and poly-L-lysine + poly-L-glutamate, respectively).

     While the phagocytosis index of the non-treated
10 macrophages reached the plateau of phagocytosis about 5 h (the difference between 4 and 5 h decreased below the insignificant level, p=1), the treated population required 8 h for the same effect. (The difference between 8 and 10 h was insignificant, p=0.058). A low but significant
15 (p=0.043) difference was obtained in the phagocytosis index after 24 h (97.3% and 94.3%, respectively).

     The time course for the ingestion index, which refers to the number of particles phagocytosed by macrophages became significant between 1 and 2 h (p=0.008). The control
20 cell population reached the plateau between 16 and 24 h (insignificant difference between the index at 12 and 24 h (p=0.841) while the treated cell population did not reach the plateau at all during the first 24 h studied. Further- more, the number of ingested particles were significantly
25 lower in the treated group at all times (p=0.043).

     Flow cytometry verified that macrophages phagocyte the test compound particles, which resulted in significant cell growth and large phagocytic vacuoles.

30 *Example 3*. Transmission electron microscopy.
     Peritoneal macrophages were harvested from two healthy non-treated animals as described above and plated on cell culture plates (Thermanox, Naperville, Il, USA). The cells were washed away after 1.5 h and poly-L-lysine +
35 poly-L-glutamate (40 μg/ml) in supplemented DMEM solution were added in sequence followed by a 24 h incubation. The

incubation medium was removed and the cells were fixed in
2.5% phosphate buffered glutaraldehyde was followed by
rinsing in Milloning's phosphate solution. Samples were
postfixed in 1% osmium tetroxide and subsequently de-
5   hydrated with graded series of ethanol, which was followed
by embedding in Araldite 502 kit. Vertical sections were
obtained with a diamond knife and stained with uranyl
acetate and lead citrate in a LKB Ultrastainer. Samples
were examined in a JEOL 1200 EX transmission electron
10   microscope (TEM).

Electron microscopy verified that macrophages
phagocyte the test compound particles, resulted in
significant cell growth, and large phagocytic vacuoles.

15   *Example 4. Scanning electron microscopy.*

Peritoneal swabs and wounds were taken from eight
treated (4) and non-treated (4) animals after one and seven
days of surgery and cell cultures were conducted as above.
The samples were fixed in 2.5% phosphate buffered glutar-
20   aldehyde at room temperature and then post-fixed in 1%
OsO_4. The samples were dehydrated in acetone, critical
point dried and sputter-coated with gold before being
studied in a LEO 420 electron microscope.

SEM data showed that mesothelial cells covered the
25   compound surface from the first day.

Example 5. Histology.

Eight animals were opened and then injected intra-
peritoneally with poly-L-lysine + poly-L-glutamate. At the
30   postoperative first, second, third, and sixth days, two
animals were sacrificed and the wounds were excised. They
were rapidly frozen and embedded, and the block obtained
was immediately cut into slices of 7 μm. The slices were
allowed to dry in dark for 30 min at room temperature and
35   were then stained with 100 μg/l 4'6'-diamino-2-phenylindole
hydrochloride (DAPI) solution for 10 min. Fluorescent

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microscopy was performed with both a FITZ and a DAPI
filter, and images were digitally merged (OpenLab,
Improvosion). Macro photo was made about the excised wounds
by using trans-illumination, mixed ambient room light, and
5   UV illumination.

The histological studies showed that the added
material was present in the wound from the first day.
Furthermore, more and more cells were detected for each day
until the matrix was completely rebuilt.

10

Example 6. Biodegradation.

Healthy nonoperated animals were treated intra-
peritoneally as in Example 1 and sacrificed after two
months.

15   No visible remains of poly-L-lysine and poly-L-
glutamate could be detected. The biodegradability is
supported by findings that at one month's follow up the
same results were obtained by using a double dose of poly-
L-lysine + poly-L-glutamate, although that caused some
20   additional adhesions related to the compound at evaluation
on the 7th day.

Comparative example 1. Biodegradation.

Aqueous solutions of 1% and 2% lysozyme, poly-L-
25   glutamate, poly-L-lysine, and poly-L-glutamate, and 0.25%
of hyaluronic acid were freshly made. Solutions of
lysozyme, polyglutamate, lysozyme + polyglutamate and
polylysine + polyglutamate were then administered to
animals as in Example 1.

30   The extent of abdominal adhesions one week after
surgery significantly decreased in the four treated groups
($p \leq 0.001$) as compared to controls. However, no significant
change in response was obtained with hyaluronic acid
($p = 0.264$). The combinations poly-L-lysine/lysozyme seemed
35   to result in an insoluble product.

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Comparative example 2. Effect of poly-L-lysine alone.

An aqueous solution of 0.5% poly-L-lysine was freshly made and administered to animals as in Example 1.

5 Such an administration of poly-L-lysine alone resulted in convulsions and death within 30 min, i.e. before they woke up from the anesthesia. The symptoms seemed to be related to the effect of opening calcium channels, plasma Ca^{++} levels being rapidly decreased.

10

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CLAIMS

1.   Use of an effective amount of a water soluble
cationic polymer, and a pharmaceutically acceptable car-
rier, in the manufacturing of a first drug and an effective
amount of a water soluble anionic polymer, and a pharma-
ceutically acceptable carrier, in the manufacturing of a
second drug, wherein said first and said second drug are to
be administered in sequence to a mammal for surface protec-
tion of exposed biological tissue surfaces.

2.   Use as in claim 1, c h a r a c t e r i z e d
in that said water soluble cationic polymer is a natural
polymer.

3.   Use as in claim 2, c h a r a c t e r i z e d
in that said natural cationic polymer is a polysaccharide,
a protein, or a polypeptide.

4.   Use as in claim 3, c h a r a c t e r i z e d
in that said polysaccharide is chitosan or a cationic
derivative of cellulose or starch.

5.   Use as in claim 3, c h a r a c t e r i z e d
in that said protein is lysozyme.

6.   Use as in claim 3, c h a r a c t e r i z e d
in that said polypeptide is a cationic polyamino acid.

7.   Use as in claim 6, c h a r a c t e r i z e d
in that said cationic polyamino acid is polylysine,
polyarginine, or polyhistidine.

8.   Use as in claim 1, c h a r a c t e r i z e d
in that said water soluble cationic polymer is a synthetic
polymer.

9.   Use as in claim 1, c h a r a c t e r i z e d
in that said first drug comprises 0.5-5 wt% of said water
soluble cationic polymer in said pharmaceutically
acceptable carrier.

10. Use as in claim 1, c h a r a c t e r i z e d
in that said water soluble anionic polymer is a natural
polymer.

11. Use as in claim 10, c h a r a c t e r i z e d in that said natural anionic polymer is a polysaccharide, a protein, a polypeptide, or a polynucleotide.

12. Use as in claim 11, c h a r a c t e r i z e d in that said polysaccharide is xanthan, alginic acid, hyaluronic acid, or polygalacturonic acid, or a salt thereof, a carragenan, or an anionic derivative of cellulose or starch.

13. Use as in claim 12, c h a r a c t e r i z e d in that said anionic cellulose derivative is carboxymethyl cellulose.

14. Use as in claim 11, c h a r a c t e r i z e d in that said polypeptide is an anionic polyamino acid.

15. Use as in claim 14, c h a r a c t e r i z e d in that said anionic polyamino acid is polyglutamate or polyaspartate.

16. Use as in claim 1, c h a r a c t e r i z e d in that said second drug comprises 0.5-5 wt% of said water soluble anionic polymer in said pharmaceutically acceptable carrier.

17. Use as in claim 6 and 14, c h a r a c t e r i z e d in that the amount of said anionic polyamino acid in said second drug exceeds the amount of said cationic polyamino acid in said first drug with reference to the number of anionic amino acid residues in relation to the number of cationic amino acid residues.

18. Use as in claim 1, c h a r a c t e r i z e d in that said pharmaceutically acceptable carrier is distilled water or a salt solution.

19. Use as in claim 1, c h a r a c t e r i z e d in that said second drug is to be administered after said first drug is to be administered.

20. Use as in claim 19, c h a r a c t e r i z e d in that said second drug is to be administered less than 5 min after said first drug is to be administered.

    21. A kit comprising:

    (d)   a first drug comprising an effective amount of a water soluble cationic polymer and a pharmaceutically acceptable carrier;

5   (e)   a second drug comprising an effective amount of a water soluble anionic polymer and a pharmaceutically acceptable carrier; and

    (f)   means for administering said first and said second drug in sequence to a mammal for surface protection

10 of exposed epithelial surfaces.

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Huvudfaxen Kassan

ABSTRACT

The invention refers to the use of an effective
amount of a water soluble cationic polymer, and a pharma-
ceutically acceptable carrier, in the manufacturing of a
first drug and an effective amount of a water soluble
anionic polymer, and a pharmaceutically acceptable carrier,
in the manufacturing of a second drug. The drugs are to be
administered in sequence to a mammal for surface protection
of exposed biological tissue surfaces.

The invention also refers to a kit comprising a first
drug comprising an effective amount of a water soluble
cationic polymer and a pharmaceutically acceptable carrier;
a second drug comprising an effective amount of a water
soluble anionic polymer and a pharmaceutically acceptable
carrier; and means for administering the drugs in sequence
to a mammal.